

A Randomized and Blinded Comparison of Three Bleeding Time Techniques: the Ivy Method, and the Simplate II® Method in Two Directions

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Summary

We compared the Ivy bleeding time method and two alternatives of the Simplate II® method (incisions in horizontal and vertical direction) with each other, with regard to the sensitivity, the specificity, the costs and the burden for the patient. In the aspirin study an aspirin induced bleeding defect was used. Seventy-two healthy volunteers were randomized to receive either 500 mg acetylsalicylic acid (ASA) or a placebo. Double blinding was maintained throughout the study. In the anticoagulation study 62 patients participated, who received oral anticoagulants (OAC) for various reasons. All participants received two bleeding time methods. The burden for the participants of each method was screened by a small standard questionnaire.

The differences in sensitivity and specificity between the three methods proved minimal. The Ivy method was more often preferred by the participants than the Simplate methods.

Since a choice on the basis of sensitivity and specificity appears not possible, we prefer the Ivy method because of lower costs and less burden.

Introduction

The bleeding time, an *in vivo* measurement of platelet function was described by Duke in 1910 (1). In order to improve Duke's test, modifications have been introduced. This has resulted in several bleeding time methods. Frequently used methods are the Ivy bleeding time, introduced by Ivy and associates (2, 3) and the Simplate II® method, based on Mielke's standardized template bleeding time (4). The Ivy bleeding time is determined by making three puncture wounds in the forearm while the Simplate II bleeding time is determined by making two incisions with a standard device. These two incisions on the forearm can be made both in a vertical (perpendicular to the antecubital crease) and a horizontal direction.

Although a strict distinction between primary hemostasis and coagulation is slightly artificial, a test for primary hemostasis is clinically useful in the diagnosis of hemostasis disorders. Desirable properties of this test are its capacity to give an indication of platelet function without being affected by the system of coagulation. We examined the three bleeding time methods in this respect: firstly, their detection performance in an aspirin induced platelet defect, secondly, their behaviour in anticoagulation. In addition, we considered the burden for the patients and the costs.

In a previous study we compared the sensitivity and reproducibility of the Ivy and Simplate II bleeding time techniques (5) and found the Ivy bleeding time method at least as sensitive for

defects of primary hemostasis as the Simplate II method. The reproducibility of both methods proved to be similar.

In that study we evaluated the Simplate II method in vertical direction, whereas the horizontal alternative of this method was not tested. Neither did we compare the methods with regard to the specificity for the process of primary hemostasis, i.e. whether the bleeding times were prolonged in hemostasis disorders not associated with platelet dysfunction. Mielke (6) and Buchanan and Holtkamp (7) assessed the influence of the direction of the incision on the bleeding time. In these studies the horizontal incision resulted in longer bleeding times than the vertical incision. Smith and associates (8) found prolonged vertical Simplate I bleeding times in patients with severely impaired secondary hemostasis (hemophilia A), whereas all Ivy bleeding times in the same patients were normal. Prolonged vertical Simplate II bleeding times have also been reported in patients on oral anticoagulant therapy (9).

The purpose of our study was to compare the Ivy and both alternatives of the Simplate II bleeding time method with each other, with regard to the sensitivity, specificity, costs and burden for the patients. In the aspirin study an aspirin induced platelet defect served as a model of impaired primary hemostasis. The anticoagulation study was performed on patients who received oral anticoagulants, i.e. patients with a hemostasis defect but with a normal platelet function. In this study we evaluated to which extent each bleeding time method was influenced by a moderate secondary hemostasis defect. The burden for the patients was assessed by a small standard questionnaire, immediately after the tests and again after 3 weeks.

Subjects, Materials and Methods

Subjects

In the aspirin study 72 healthy volunteers participated, ranging in age from 21 to 47 years. None of the participants had ingested acetylsalicylic acid (ASA) or other drugs known to interfere with platelet function for at least ten days before the study. None of the participants reported any relatives known with a bleeding tendency.

In the anticoagulation study 62 patients participated, who received oral anticoagulants for various reasons. Their ages ranged from 38 to 65 years. Inclusion criteria for this part of the study were: stable level of long term anticoagulation and an INR (international normalized ratio) >3.0 at a previous visit to the Leiden Thrombosis Service. Patients who used ASA or other drugs known to interfere with platelet function were excluded. The study was approved by the medical ethics committee of our institution and all participants gave informed consent.

Bleeding Time Techniques

After shaving the volar surface of the forearm when necessary the volar surface was cleansed with diethyl ether and allowed to dry. Venostasis was achieved with a standard blood pressure cuff inflated to 40 mmHg throughout the procedure. Three puncture wounds (Ivy) or two incisions (Simplite II) were made at a distance of approximately 5 cm

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from the antecubital fossa on the lateral aspect of the volar surface of the forearm in an area free of superficial veins. Incisions were made either in horizontal (parallel to the antecubital crease) or vertical directions (Fig 1). A stopwatch was started at the appearance of the first drop of blood.

For the Ivy bleeding time we used a sterile lancet (Becton Dickinson and Company, Rutherford, NJ) with a blade depth of 2.5 mm and a blade width of 1.5 mm. The Simplate II device (General Diagnostics, Morris Plains, NJ) made two incisions, each 6 mm long and 1 mm deep. Whatman No. 1 filter paper was used to blot the blood from the puncture wounds or incisions at 15- and 30-s intervals, respectively. Care was taken not to disturb the wounds. The end point was defined as the moment when blood had stopped appearing from the wound and the filter paper no longer turned red. Adhesive tape was firmly applied to the incisions of the Simplate II in order to close the gaps of the wounds and to limit scar formation. The bleeding time was expressed as the average time of the three punctures (Ivy) or two incisions (Simplat II), rounded to the nearest quarter (Ivy) or half minute (Simplat II).

All bleeding times were performed by the authors RS and AS after intensive training prior to the study. The aspirin study was carried out at the clinical coagulation laboratory of the University Hospital Leiden and the anticoagulation study at the Leiden Thrombosis Service, the regional anticoagulation monitoring institution.

Design of the Aspirin Study

The 72 healthy volunteers were randomized into three groups, each consisting of 24 volunteers. The subjects of the first group received an Ivy bleeding time in one arm and a Simplate II bleeding time in vertical direction (Simplat II_{vertical}), in the other arm. The subjects of the second group received an Ivy bleeding time in one arm and a Simplate II bleeding time in horizontal direction (Simplat II_{horizontal}), in the other arm. The subjects of the third group received a Simplate II bleeding time in both arms, in vertical direction in one arm and in horizontal direction in the other arm.

In order to achieve an equal distribution by age and sex in the three groups, a stratified randomization was carried out according to the minimization method (10).

In each group 12 volunteers were given 500 mg of ASA orally whereas the other 12 volunteers received a placebo. Two hours later the bleeding times were performed. ASA and placebo were allocated at random using a random number table. Double blinding was maintained throughout the study. Each group was divided into 4 subgroups consisting of 6 volunteers. In this way the investigators (RS and AS) performed both bleeding time methods in each group equally often on the left and right arm (Table 1).

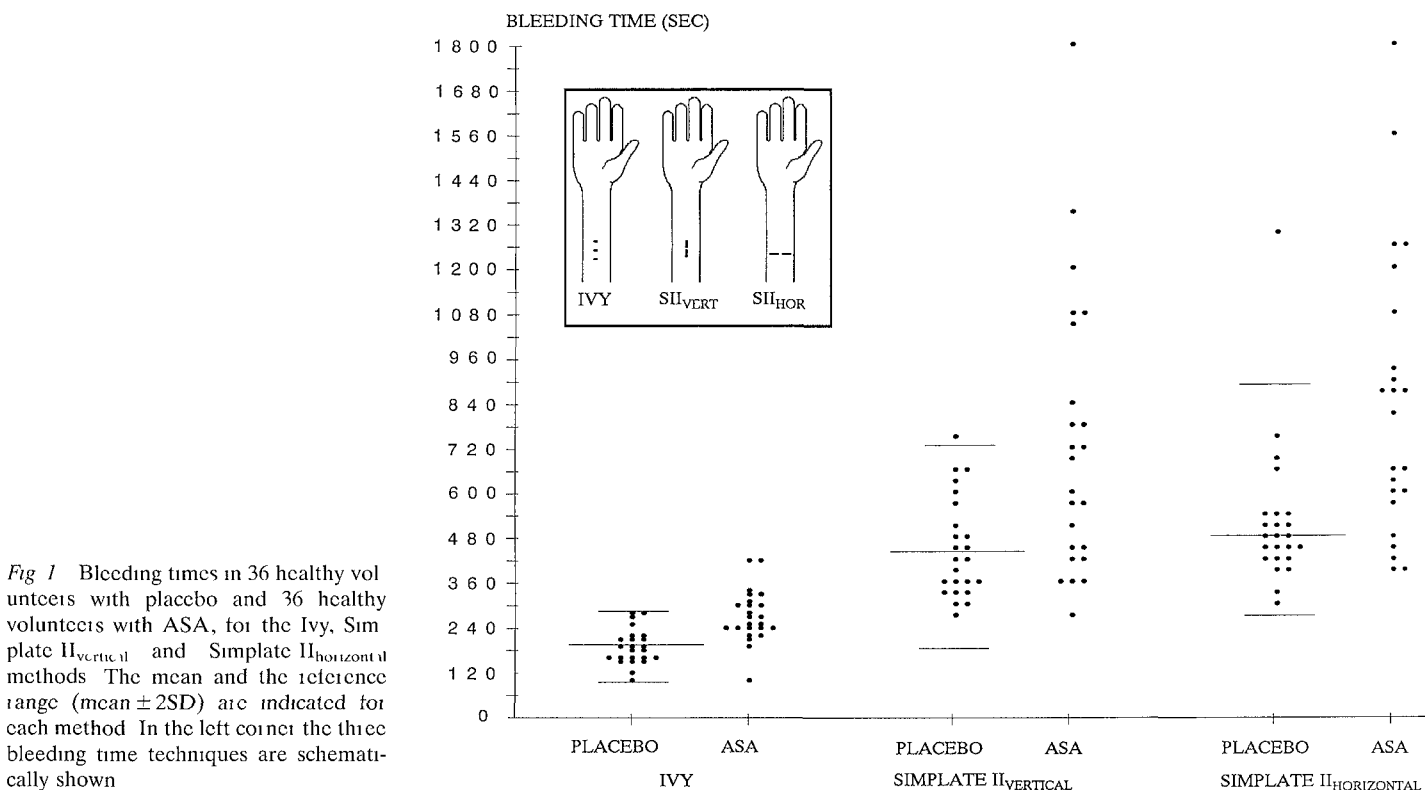
When a volunteer came to the laboratory the first investigator performed one of both bleeding time methods on one arm. The volunteer was instructed not to inform the second investigator about the result obtained by the first investigator. The second investigator then performed the other bleeding time method on the other arm. Care was taken to start equally often with each bleeding time method. Directly after performance of the two bleeding time methods and again three weeks later the volunteers were asked which method they preferred.

Table 1 Design of the study

Group	Subgroup	Method performed by RS	Arm	Method performed by AS	Arm
Ivy-SII _{vert}	A	Ivy	Left	SII _{vert}	Right
	B	Ivy	Right	SII _{vert}	Left
	C	SII _{vert}	Left	Ivy	Right
	D	SII _{vert}	Right	Ivy	Left
Ivy-SII _{hor}	A	Ivy	Left	SII _{hor}	Right
	B	Ivy	Right	SII _{hor}	Left
	C	SII _{hor}	Left	Ivy	Right
	D	SII _{hor}	Right	Ivy	Left
SII _{vert} -SII _{hor}	A	SII _{vert}	Left	SII _{hor}	Right
	B	SII _{vert}	Right	SII _{hor}	Left
	C	SII _{hor}	Left	SII _{vert}	Right
	D	SII _{hor}	Right	SII _{vert}	Left

Ivy: bleeding time according to Ivy.

SII_{vert}, SII_{hor}: bleeding time with Simplate II device in vertical and horizontal direction respectively.



Design of the Anticoagulation Study

The 62 patients were randomized into three groups as in the sensitivity study. Both in the first (Ivy-Simplate II_{vertical}) and the second (Ivy-Simplate II_{horizontal}) group, 21 patients participated. In the third group (Simplate II_{vertical}-Simplate II_{horizontal}) 20 patients participated. The randomization was carried out according to the minimization method, to ensure equal age and sex distribution over the three groups. In all aspects

the design of the anticoagulation study was equal to the design of the aspirin study except that the patients received neither ASA nor placebo and that the intensity of anticoagulation (expressed in INR) was determined after the bleeding times.

Statistical Analysis

Use of the minimization method justifies the pooling of all bleeding time determinations of each method for statistical analysis. The normality of the underlying distributions of the bleeding times was tested with the Kolmogorov-Smirnov test (11). For comparison of proportions a chi-square test was used. For comparison of the means of the bleeding times in the ASA and placebo groups a two-sample *t* test was used.

When tests do not have binary outcomes but rather have a continuum of values, the true positive fraction (sensitivity) and the false positive fraction (1 minus specificity) vary with the value selected as the cut-off point. The sensitivity is defined as the proportion of those with the condition who have a positive test result and the specificity as the proportion of those without the condition who have a negative test result. We graphically visualized the effect of changes in the cut-off point on test performance, i.e. sensitivity and specificity by using a receiver operating characteristic (ROC) curve (12-14), a plot of the true positive fraction against the false positive fraction for varying cut-off points.

Results

Aspirin Study

After stratified randomization the sex and age distributions in the three groups were similar, each group consisted of 12 men and 12 women, the mean ages were 27, 25 and 27 years. The bleeding times for each method after ingestion of ASA and placebo are shown in Fig 1. The bleeding times determined by the Ivy and Simplate II_{vertical} method were normally distributed. The distribution of the bleeding times determined by the Simplate II_{horizontal} method was log-normal. With each of the three techniques, we found longer average bleeding times in the ASA group than in the placebo group.

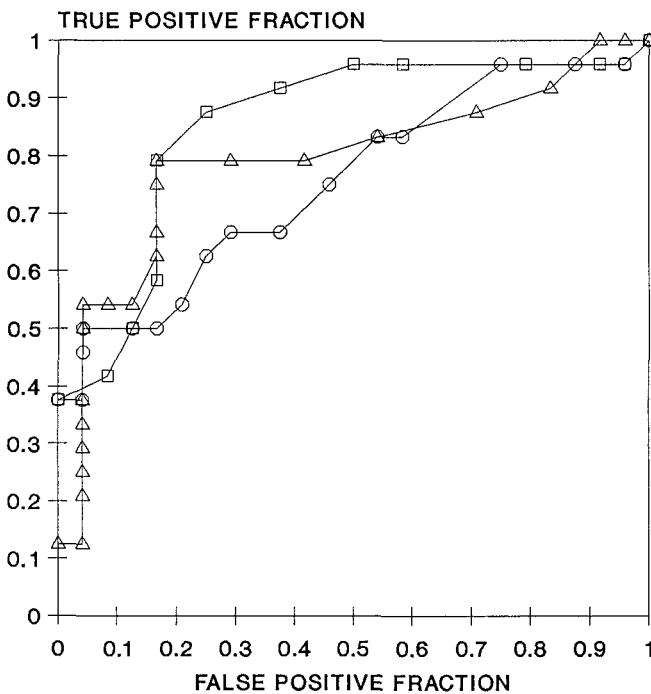


Fig 2 A plot of the true positive fraction (sensitivity) against the false positive fraction (1 minus specificity) for the Ivy (□), the Simplate II_{vertical} (○) and the Simplate II_{horizontal} (△) methods, based on the findings in 72 healthy volunteers who took ASA or placebo.

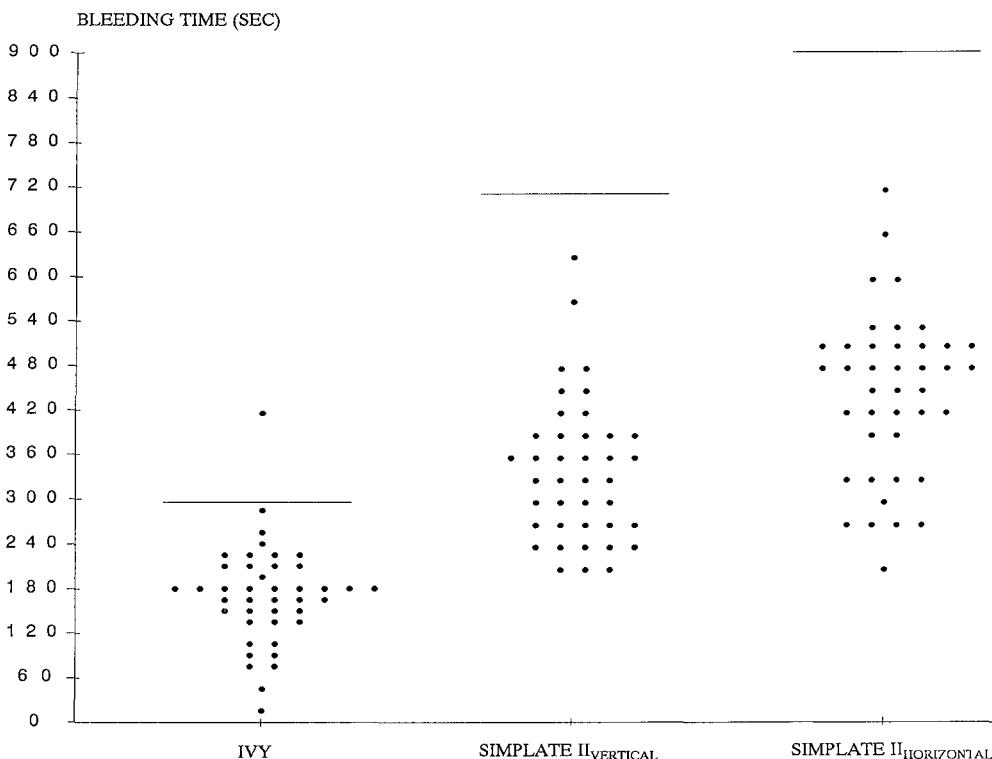


Fig 3 A plot of the bleeding times in 62 anticoagulated patients for the Ivy, Simplate II_{vertical} and Simplate II_{horizontal} methods. The cut-off point for each method is indicated by the horizontal line.

Table 2 Preference of all study subjects

Method preferred	Study groups		
	Ivy-SII _{vert}	Ivy-SII _{hor}	SII _{vert} -SII _{hor}
Ivy	18	22	n. a.
SII _{vert}	7	n. a.	7
SII _{hor}	n. a.	1	5
No preference	18	17	24
Total	43	40	36

The preference of all subjects after 3 weeks have passed. Eighty-nine percent of all participants responded to our inquiry. n. a.: not applicable.

The test results of the 24 healthy volunteers who took a placebo served to find a reference range for each method. The limits of normal were set at the mean plus or minus two times the standard deviation (SD) and rounded to the nearest quarter minute (Ivy) or half minute (Simplate II). For the Simplate II_{horizontal} method this was done after logarithmic conversion. The mean of the Ivy bleeding times was 3.2 min with a reference range of 1.6–4.75 min (mean \pm 2SD). The mean of the Simplate II_{vertical} bleeding times was 7.5 min with a reference range of 3.0–12.0 min. The mean of the Simplate II_{horizontal} bleeding times was 8.2 min with a reference range of 4.6–15.0 min.

In the Ivy, Simplate II_{vertical} and Simplate II_{horizontal} groups, the proportions of individuals with prolonged bleeding times after ASA ingestion (cut-off point: mean \pm 2SD) were similar. The sensitivity (true positive fraction) for the Ivy was: 38% (9/24), for the Simplate II_{vertical}: 38% (9/24) and for the Simplate II_{horizontal}: 33% (8/24). To avoid a comparison of the test performance of the bleeding time methods on the basis of one single cut-off point for each method, we constructed ROC curves for the three bleeding time methods (Fig. 2) by use of the data presented in Fig. 1. In general, better test performance is indicated by a ROC curve that is higher and more to the left in the ROC space (13). Inspection of the three curves confirms similar sensitivity and specificity of the three tests.

Anticoagulation Study

Stratified randomization of the 62 anticoagulated patients resulted in similar sex ratios and mean ages in the three groups: first group (Ivy-Simple II_{vertical}): 17 men, 4 women, mean age 54 years; second group (Ivy-Simple II_{horizontal}): 18 men, 3 women, mean age 55 years; third group (Simple II_{vertical}-Simple II_{horizontal}): 17 men, 3 women, mean age 57 years. The bleeding times determined by each method are presented in Fig. 3. The intensity of anticoagulation of the patients ranged from INR 1.5 to INR 8.0 with a mean of INR 3.6.

The proportions of individuals with prolonged bleeding times (cut-off points as determined in the aspirin study) in the Ivy, Simple II_{vertical} and Simple II_{horizontal} groups were similar: Ivy: 2% (1/42); Simple II_{vertical}: 0% (0/41); Simple II_{horizontal}: 0% (0/41). The mean bleeding time (compared to the bleeding times in the placebo groups of the aspirin study) was not prolonged for any of the three methods.

Judgment of Methods by the Subjects

Table 2 presents the methods preferred by the subjects a few weeks after performance of the bleeding times, in both the aspirin and the anticoagulation study. Immediately after the tests had been performed, many had not yet formed an opinion; after some weeks had passed, however, most of the subjects (48%) in both

Ivy-Simple II groups preferred the Ivy method, whereas only 10% preferred one of the both Simple II techniques, of which the Simple II_{horizontal} was least popular.

The main reason for a higher preference of the Ivy method was the absence of scar formation. Twenty-three percent of all subjects who underwent a Simple II reported scar formation, but none of those who underwent an Ivy bleeding time.

Discussion

The bleeding time is one of the most used hemostatic tests. In order to improve the test several methods have been developed. Rodgers and Levin (17) have recently reexamined hundreds of reports concerning the bleeding time. They found no evidence that advances in standardization of the technique resulted in a better detection performance. No differentiation was made, however, between the Simple in vertical and horizontal direction.

In our study, in which we have applied rigorous methodologic standards, the Ivy and Simple II (horizontal and vertical) bleeding time methods proved equally sensitive to an aspirin induced defect of platelet function. This is in accordance with the findings of Buchanan and Holtkamp (7) who reported the Simple I to be equally insensitive to an aspirin induced defect regardless of the direction of the incision. Mielke (6), however, found the best detection performance with an incision in the horizontal direction, when using a template device.

In our previous study (5) the Ivy technique was more sensitive than the Simple II_{vertical} in the statistical sense, but the difference was small and not clinically relevant. We therefore concluded that the Simple II_{vertical} and the Ivy had similar detection performance, as was reported previously by Bain et al. (18). The results of our present study confirm our impression that any relevant difference between the different bleeding time methods, the Simple II_{horizontal} included, is unlikely.

The reference ranges we found were high compared to most other studies, our previous study included, especially for both Simple II techniques, although similar high reference ranges have been reported previously (18). This wide range of reference ranges may be caused by age differences (15, 16), variability in performance and interpretation of the techniques (19), and variability in the Simple II device itself: variability between different lotnumbers of the Simple II device has recently been reported (20).

In the anticoagulation study none of the methods proved to be sensitive for a moderate secondary hemostasis disorder caused by oral anticoagulants. In a previous study on patients with anticoagulation, the average bleeding time was significantly prolonged (9). In that study the upper limit of normal and the fraction of individuals with prolonged bleeding times were not reported, and it has to be noted that this fraction can be low even though the mean bleeding time is significantly prolonged. Nevertheless in our study no prolongation of the bleeding times could be found. An explanation for this difference could be that our volunteers who received a placebo were younger than the anticoagulated patients. The absence of prolonged bleeding times in patients with a moderate secondary hemostasis disorder caused by oral anticoagulation may not be extrapolated to patients with another or severe secondary hemostasis disorder.

As a choice between the three methods on the basis of sensitivity and specificity appears not possible, the burden for the patients and the costs should play a major role. The participants in our study generally preferred the Ivy method. The Simple II method is considerably more expensive than the Ivy method. This is not only because the device costs approximately 27 times as much as the Ivy lancet, but also because the test is far more time

consuming. To indicate the difference in performance time, the summed bleeding times of each method in both studies are: Ivy 5.1 h, Simplate II_{vertical} 11.8 h, Simplate II_{horizontal} 14.4 h.

Since the three tests in this study show similar sensitivities and specificities, we conclude that costs and burden should play a major role in the choice between the three bleeding time techniques and therefore we prefer the Ivy method.

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REFERENCES

- 1 Duke WW. The relation of blood platelets to hemorrhagic disease: description of a method for determining the bleeding time and coagulation time and report of three cases of hemorrhagic disease relieved by transfusion. *JAMA* 1910; 55: 1185-92.
- 2 Ivy AC, Shapiro PF, Melnick P. The bleeding tendency in jaundice. *Surg Gynecol Obstet* 1935; 60: 781-4.
- 3 Ivy AC, Nelson D, Bucher G. The standardization of certain factors in the cutaneous "venostasis" bleeding time technique. *J Lab Clin Med* 1941; 26: 1812-22.
- 4 Mielke CH Jr, Kaneshiro MM, Maher JA, Weiner JM, Rapaport SI. The standardized normal Ivy bleeding time and its prolongation by aspirin. *Blood* 1969; 34: 204-15.
- 5 Koster T, Cackebek-Peculnick KMJ, Briet E. A randomized and blinded comparison of the sensitivity and the reproducibility of the Ivy and Simplate II® bleeding time techniques. *Am J Clin Pathol* 1989; 92: 315-20.
- 6 Mielke CH Jr. Aspirin prolongation of the template bleeding time: influence of venostasis and direction of incision. *Blood* 1982; 60: 1139-42.
- 7 Buchanan GR, Holtkamp CA. A comparative study of variables affecting the bleeding time using two disposable devices. *Am J Clin Pathol* 1989; 91: 45-51.
- 8 Smith PS, Baglin R, Meissner GF. The prolonged bleeding time in hemophilia A: a comparison of two measuring techniques and clinical associations. *Am J Clin Pathol* 1985; 83: 211-5.
- 9 Maionigi F, Biondi G, Sorano GG, Mameli G, Conti M, Mamusa AM, Cadoni MC, Balestracci A. Bleeding time is prolonged during oral anticoagulant therapy. *Thromb Res* 1990; 59: 905-12.
- 10 Pocock SJ. *Clinical Trials: a Practical Approach*. John Wiley and Sons, Chichester 1983; pp 84-6.
- 11 De Jonge H. *Inleiding tot de medische statistiek*. Nederlands Instituut voor Preventieve Geneeskunde, Leiden 1958; pp 217-20.
- 12 McNeil BJ, Keeler E, Adelstein SJ. Primer on certain elements of medical decision making. *N Engl J Med* 1975; 293: 211-5.
- 13 Metz CE. Basic principles of ROC analysis. *Semin Nucl Med* 1978; 8: 283-98.
- 14 Turner DA. An intuitive approach to receiver operating characteristic curve analysis. *J Nucl Med* 1978; 19: 213-20.
- 15 Jørgensen KA, Dyerberg J, Olesen AS, Stoffensen E. Acetylsalicylic acid, bleeding time and age. *Thromb Res* 1980; 19: 799-805.
- 16 Macpherson CR, Jacobs P. Bleeding time decreases with age. *Arch Pathol Lab Med* 1987; 111: 328-9.
- 17 Rodgers RPC, Levin J. A critical reappraisal of the bleeding time. *Semin Thromb Hemostas* 1990; 16: 1-20.
- 18 Bain B, Forster T, Baker A. An assessment of the sensitivity of 3 bleeding time techniques. *Scand J Haematol* 1983; 30: 311-6.
- 19 Poller L, Thomson JM, Tomenson JA. The bleeding time: Current practice in the UK. *Clin Lab Haematol* 1984; 6: 369-73.
- 20 Machereel P, Sulzer I, Furlan M, Lammle B. Warning: Simplate II - Lack of standardization in standardized bleeding time devices. *Thromb Haemostas* 1990; 64: 605.

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